A PROSPECTIVE NON COMPARATIVE STUDY ON EFFICACY AND SAFETY OF ENZYMATIC BURN WOUND DEBRIDEMENT USING BROMELAIN DERIVED DEBRIDING AGENT ESCHALYSE

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CERTIFICATE

Certified that the dissertation entitled **"A PROSPECTIVE NON COMPARATIVE STUDY ON EFFICACY AND SAFETY OF ENZYMATIC BURN WOUND DEBRIDEMENT USING BROMELAIN DERIVED DEBRIDING AGENT ESCHALYSE"** is a bonafide work done by **Dr.M.RAJKUMAR,** Postgraduate, Department of Burns, Plastic and Reconstructive Surgery, Kilpauk Medical College, Chennai under my guidance and supervision in partial fulfillment of the regulation of the Tamil Nadu Dr.M.G.R. Medical University for the award of M.Ch (Plastic surgery) Branch-III during the academic period of August 2004 to August 2007.

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INTRODUCTION

One of the major characteristics of burns is the formation of an eschar, which is made up burned and traumatized tissue. The presence of the eschar that covers the entire injured area prevents accurate diagnosis of the burns depth and may lead to the extension of injury to neighboring, originally undamaged tissues. The eschar also serves as a medium for bacterial growth, and is therefore a source of infection, contamination and sepsis. As a result, prompt removal of the eschar is imperative to the healing of burns[1-5].

The current method of choice for burn debridement is surgical tangential excision as advocated by Janzekovic in 1970. While effective, surgical debridement has several major disadvantages. Tangential excision is non-selective and may sacrifice healthy surrounding tissues, often converting a partial thickness burn into a full thickness defect[1,6-8]. Furthermore, surgical excision is painful and exposes patients to the risks of repeated anesthesia and significant bleeding. Enzymatic debridement has been suggested in the past, however the agents used have had several drawbacks. In particular, most enzymatic agents require prolonged and repeated exposures in order to achieve sufficient debridement often necessitating further surgical or chemical debridement. Furthermore, repeated application, especially when using moist occlusive dressings for extensive periods of time, may result in local infection and promote systemic spread of the infectious process[9-13].

The ideal debridement agent or method should have the following attributes:

1. Safety i.e., without any systemic and local adverse effects.

2. Selectivity: resulting in removal of the necrotic eschar without affecting the surrounding viable tissue, thus permitting accurate diagnosis of the extent of the original damage.

3. Effective : removing the entire eschar, preferably in a single application.

4. Rapid: resulting in rapid reduction of the infection risk and permitting sequential debridement of large areas over a short time span.

5. Simple to use and cost effective.

Bromelain is a well-known group of enzymes extracted from pineapple fruits or stems. It contains more than 50 different components and is widely used as an over-the-counter food additive and is also used in the cosmetic industry. The late, Drs.Klein and Houck,[14-15] attempted to debride burn eschars with bromelain. They initially used commercially available lyophilized preparations achieving good but inconsistent results[16-18]. Further work led to the development of a proprietary extraction method that purified active ingredients from the crude bromealin to obtain a highly effective debriding mixture that they called "Eschalyse"[19]. The developers claimed that the effective action of Eschalyse was due to the synergistic activity of its various components.

AIM OF THE STUDY

The aim of the current study was to evaluate the efficacy of burn wound debridement using the Eschalyse enzyme preparation as well as to evaluate its

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REVIEW OF LITERATURE

Improvements in burn care originated in specialized units specifically dedicated to the care of burned patients. The interactive multidisciplinary team has proven to be the least expensive and most efficient method of treating major burn injury, of which the initial acute care is only a small part of the total treatment. Burn patients often requires years of supervised rehabilitation, reconstruction, and psychosocial support. Omission of any step in the treatment regimen by any of the burn team members, including the burn surgeon, nurses, therapists, nutritionists, or psychosocial support staff, can result in less than optimal outcomes.

EPIDEMIOLOGY

A study conducted in United States says that approximately 1.1 million individual annually are burned seriously enough to seen health care; about 45,000 die. More than 90% of burns are preventable; nearly one half are smoking related or due to substance abuse.[23,24]

The burn size associated with a 50% mortality rate has increased from 30% of the total body surface area (TBSA) to greater than 80% TBSA in otherwise healthy young adults.[25,26] The quality of burn care is no longer measured only by survival, but also by long-term function and appearance. Although small burns are not usually life threatening, they need the same attention as larger burns to achieve optimal outcomes.

As with other forms of trauma, burns frequently affect children and young adults. In children under 8 years of age, the most common burns are scalds, usually from the spilling of hot liquids.[27] In older children and adults, the most common burns are flame-related, usually the result of house fires, the ill-advised use of flammable liquids as accelerants, or smoking – or alcohol – related.[28] Chemicals or hot liquids, followed by electricity, and then molten or hot metals most often cause work-related burns.[29]

NORMAL SKIN FUNCTION

The skin is the largest single organ of the body. It conforms to body contours, flexes with muscular action and is sufficiently elastic to allow for drastic changes in body contours. Through a variety of sensors, the skin provides a perception of the environment, body positions, heat, texture and pressure, even in the absence of the other senses. The bi-layer composition of the skin provides an external water repellent and internal vapor barrier and is the anchor tissue for all dermal appendages. This elastic organ protects internal structures from injury and is an effective barrier to the instruction of dirt, dust and microorganisms. Through the controlled excretion of water and solutes, body heat is dissipated, and in concert with the hypothalamus, provides the human organism the means to maintain a stable core temperature in a variety of external environments. Additionally sunlight stimulates the skin to produce Vitamin D, which is an essential nutrient for normal absorption and utilization of calcium and phosphorus.

Scattered throughout the dermis are three types of connective cells; mast cells which secrete histamine and heparin, phagocytic histiocytes and the reparative fibroblast. Burn injury stimulates each of these cells producing a series of local and systemic changes.

BURN DEPTH

The depth of burn varies depending on the degree of tissue damage. Burn depth is classified into degree of injury in the epidermis, dermis, subcutaneous fat, and underlying structures.

Depths

First Degree	**Injury localized to the epidermis**
Superficial second degree	Injury to the epidermis and superficial dermis
Deep second degree	Injury through the epidermis and deep into the dermis
This degree	Full-thickness injury through the epidermis and dermis into the subcutaneous fat
Fourth degree	Injury through the skin and subcutaneous fat into underlying muscle or bone

BURN SIZE

Determination of burn size estimates the extent of injury. Burn size is generally assessed by the “rule of nines”. In adults, each upper extremity and the head and neck are 9% of the TBSA. The lower extremities and the anterior and posterior trunk are 18% each, and the perineum and genitalia are assumed to be 1% of the TBSA. Children have a relatively larger portion of the body surface area in the head and neck, which is compensated for by a relatively smaller surface area in the lower extremities. Infants have 21% of the TBSA in the head and neck and 13% in each leg, which incrementally approaches the adult properties with increasing age.

MECHANISM OF INJURY

Thermal Burns

Heat denatures cellular proteins resulting in a cell coagulation necrosis. The severity of the injury is related to the intensity of the heat, i.e., temperature, the length of exposure, and the conduction capacity of the skin at the site of the injury.[31-34]

Burns caused by hot liquids are usually partial thickness unless prolonged contact with the causative agent occurs although the relative thinness of the skin of the elderly and young children may result in deeper injuries than initially suspected. Full thickness injuries are most frequently the result of direct flames, boiling water or grease, molten metals, synthetic material, and other substances, likely to adherent to the skin, such as tar or asphalt. Immediate cooling of these adherent substances will reduce the amount of tissue damage resulting from such exposure.

Chemical injuries

The extent and severity of a chemical injury is dependent upon the agent's strength or concentration, quantity, physical state, duration of contact and penetrating power. Injuries caused by chemical exposure differ from thermal injuries in the rate at which tissue destruction occurs and the length of time necrosis continues. Tissue necrosis will continue to occur as long as the undiluted agent remains on the skin or until the caustic agent is chemically expended.

The scientific evidence of the exact mechanism of chemical injury is ill-defined and dependent upon the specific chemical agent. In general, agents can be classed into five groups, based on the manner in which they coagulate proteins. These are: (1) oxidizing agents, such as sodium hypochlorite, (2) corrosives, such as phenol and lye, (3) salt forming agents such as formic, tannic and hydrochloric acids, (4) dessicants such as sulfuric acid and (5) vessicants such as DMSO and nitrogen mustard. In addition to cutaneous injury, some agents cause systemic toxicity with symptoms such as hypocalcemia, hepatic necorsis and renal tubular damage.

Alkalis penetrate deeply, causing liquefaction necrosis and fat saponification. Rumack reported temperatures of 96°C in cat esophagi after lye ingestion, contributing heat damage to the chemical injury. Acids, in contrast, are potent dessicants and cause tissue injury by cellular dehydration and lysis in addition to local heat generation. Acids do not penetrate as well as alkalis due to the protective eschar formation from the heat generated protein coagulation.

Electrical injuries

Electrical burns produce a visible cutaneous injury which belies the actual damage sustained. The extent of injury is dictated by the type of current (direct vs indirect), the current voltage and amperage, resistance of the body parts through which current passes and the duration of contact. Most high voltage current tends to take a direct path from the point of contact to ground whereas lower voltage alternating current more frequent results in cardiac arrest and death. A 15 mill ampere or greater and death. A 15 mill ampere or greater current will produce tetanic muscle contractions making it impossible release the contact until heat generation results tissue carbonization.

Electricity flows along the path of least resistance, which in the body is along the nerves and blood vessels. Different tissues offer different resistances, ranging from least to greatest they are nerve, blood, muscle, skin, tendon, fat and bone. The more resistant the tissue, the greater the heat produced as electiricty passes through it. Perspiration or moisture reduce skin surface resistance by 12-25 fold[38] however, as the skin is the usual point of contact, entry and exit wounds occur at the point of electrical contact and the site of grounding.

True electrical injuries are produced when electricity passes through the body after contact with a conductor. The characteristic entry and exit wounds represent deep issue destruction. The body part(s) through which the electricity passed may appear viable but muscle tissue death is progressive and may not be accurately assessed until 4 – 5 days following injury. The arc burn is caused by current coursing external to the body, jumping from the contact point to the ground and is most frequently associated with high tension sources. Characteristically, these injuries have scattered sports of injury due to the high temperature of the arc. Flame injuries may also result from electrical contact when the electricity sparks or arcs to clothing igniting them.

Radiation injury

Ionizing radiation i.e., the type produced by X-rays, radioisotopes and classic radioactive substances, causes injury to the body through its ability to penetrate cells and deposits energy within them. When sufficiently intense, ionizing radiation kills cells through inhibiting mitosis. The sensitivity of cells to radiation damage varies considerable with the different stages of cell life. In addition to cutaneous injury, excessive exposure to radiation can produce life-threatening systemic effects such as extensive hemorrhage, aplastic anemia and destruction of lymphatic tissue. These injuries characteristically result in full thickness dermal injury and due to the extensive cellular damage, are slow to heat.

Pathophysiology of the burn wound

Three concentric zones of burn injury have been described. The central zone of coagulation represents the direct heat damage, consisting of protein coagulation with complete cessation of blood flow through arterial and venous channels, which results in irreversible tissue death. Additionally, if a large body surface area is severely damaged, the reduction of circulating volume results in tissue hypoxia, with a concomitant shift to anaerobic metabolism and lactic acid accumulation, causing further structural damage. If this zone extends below the dermal appendages, a full thickness injury results whereas if lies above the dermal appendages, a partial for spontaneous health. The zone of coagulation is surrounded by the zone of stasis, characterized by oxygen and nutrient starved tissue. If the area of stasis is protected from infection and dessication, the area may ultimately survive and heal. The zone of hyperemia surrounds the other areas and is the site of minimal cell involvement early spontaneous recovery, if provided with appropriate cases.

Severe burn injury produces venous stasis, microthrombi formation and vascular endothelial sloughing. As the blood’s viscosisty changes, white cells marginate and red cell rouleaux formation occurs, creating a physical barrier between the phagocytes and any bacteria present. Additionally, this barrier compromises oxygen, antibody and phagocyte delivery to the burned tissue interface.

Using light staining techniques electron microscopy and various histochemical stains, deCamara et al[41] obtained structural as well as cellular information on the nature of thermal injury. After producing an epidermal scald burn in guinea pigs, they observed coagulative necrosis of epidermal cells with epidermal – dermal separation within 2 hours. Loss of basement membrane continuity and dermal edema, with disruption of superficial dermal capillaries, was noted at eight hours post injury. A complete inflammatory response was present at 96 hours, with dermal infiltration by polymorphonuclear leukocytes. With the documentation of the progressive nature of the burn would and the realization that the height of local damages does not occur immediately after the burn, the potential exists for reversing or preventing the burn wound progression.

Inflammatory Response

The capillaries and venules in the burn wound become highly permeable to fluid, electrolytes and proteins in the area of burn wound. Netsky and Leiter in 1943 documented the passage of horse serum protein across the capillary endothelium of the dog following thermal injury and the results were confirmed with radioactive dyes and Evans blue.[42,43] Massiha and Manafo[44] demonstrated that venous circulation was more compromised than arterial in the zone of stasis. Polymorphonuclear leukocytes (PMN)adhere to the vessel walls and platelet aggregation occurs, increasing venous resistance and causing post burn edema.[45] In server thermal injury, micorthrombi form and occlude the microcirculation, incurring further cell death. Chemical mediators released from the site of injury are responsible for the development of the typical inflammatory response.

The inflammatory response to thermal injury results in rapid and dramatic edema formation. This transcapillary transport has been extensively studied by Arturson and colleagues[46] who attribute the phenomena to the combined effects of three mechanisms: (1) dilation of resistance vessels resulting in increased transcapillary filtration pressure, (2) increased extravascular osmotic activity in damaged tissue and (3)increased microvascular permeability to macromolecules. The combined effects of these alterations result in rapid loss of vascular fluid into the interstitium. A large amount of literature exists on morphological ultra structure changes following thermal injuries which could account for the fluid shift. Many authors have suggested the open endothelial intercellular junctions allow fluid and protein to escape[47,48] and it appears that the junctional openings occurs in venules first.

Heat denatured proteins stimulate the acute inflammatory process by activating the complement cascade. The activated complement system facilitates liberation of various permeability factors at the site. Histamine is one of the first mediators released and elevated levels of histamine have been measured in lymph drainage following thermal injury.[49] Other investigation have documented high levels of prostaglandins in wound secretions and blister fluid, principally PGE-1, PGE-2 and PGF-2a.[50,51,51] Recently the endoperoxides and thromboxanes, which cause platelet aggregation and contract vascular and airway smooth muscle have been found and quantitated in burn blister fluid.[52]

Polymorphonuclear neutrophil leukocytes stick to the wall in large numbers within minutes of thermal injury and their oxidative metabolism suddenly increases during phagocytic activity.[53,54] NADPH oxidases present in the cell wall reduce oxygen to superoxide radical and hydrogen peroxide, which kill the bacteria or destroy the phagocytised material. Cell damage is prevented by the presence of superoxide dismutase (SOD) and catalyse in the cytosol. In the burned tissued, the overwhelming phagocytic response results in relative deficiencies of SOD and catalyse and excess free radicals damage the cell walls, releasing arachonidonic acid and stimulating the formation of prostaglandins, endoperoxides, prostacyclin, thromboxane, A2 and B2, resulting in cell contraction and macromolecular leakage. A significant reduction in post burn edema has been observed following delivery of SOD and activate catalyse in experimental rat models.[55]

Water and heat loss

The burn wound exudates and evaporate continuously from the burn wound surface creating a hyperosmotic zone which attracts more water from the underlying vital tissues, establishing a vicious cycle.

Every square meter of burn surface area results in the loss of 3750 mls of water and each liter of water requires 580 kcal of energy to evaporate.[56] This excessive loss of heat from the body is further enhanced by decreased insulation afforded by damaged skin, increasing radiation heat loss. A massive catabolic response, with the release of catecholamines, glucagon and relative insulin inefficiency results in proteolysis, lipolysis, gluconeogensis and increased substrate flow to liver.[57-61] These changes are clinically observed as decreased body weight, excessive nitrogen loss or body wasting and increased oxygen consumption. This hyper metabolic response can be modified to a certain extent by increasing the ambient temperature[62] and the proper nutritional support.[63]

Alteration in host response and sensistivity to infection

Several factors are responsible for the decreased resistance to infection in the burn wound. These include:

1. Epidermal loss exposes vital tissues to bacterial colonization and invasion.

2. The burn eschar and exudates are an ideal media for growth of bacteria

3. Coagulative necrosis and loss of circulation prevents humoral defences from reaching the affected tissue and

4. Alterations in humoral antibody activity.

Macromolecular leakage into the burned areas, catabolism and reduced immunoglobulin synthesis result in a decreased concentration of all individual immunoglobulin levels, reducing direct and indirect neutralization activity or triggering of the complement cascade. Specific cell mediated immunity is down regulated as seen by altered T4/T8 ratio with an increase in suppressor cells and natural killer cells (NK cells) number and function decreases. Further research to study the alteration in cell mediated is necessary to understand their function.

Non specific cellular immune response

After the burn, the consumption of complement reduction serum immunoglobulin concentrations and multiple defects in polymorphonuclear leukocyte functions appear. They are: (1) decreased chemotaxis (2) random migration, (3) reduced phagocytosis rates and (4) impaired bactericidal capacity. Consumption of stimulatory factors[47] and or appearance of inhibitors following burns injury is considered responsible for reduced chemotaxis and the occurrence of random migration. Defects in killing ability of gram positive organisms by polymorphonuclear leukocytes have been documented by many authors[64,65] so it is that reduced ability to phagocytize the particles. The reticuloendothelial system as well as bone marrow granulopoiesis,[66] are depressed in burn patient, reducing factors influencing the opsonization of foreign microorganisms.

Eschar separation and wound healing

As the acute, non-specific inflammatory response reaches its peak two or three days following thermal injury, the small, still functional vessels regain their integrity and reabsorb the extravased fluid. Marcrophages appear in the area and the invading colonized bacteria secrete collagenase and other proteases, starting the separation (lysis) of dead from live tissue. The more infected the wound, the quicker eschar separation occurs. In deeper burns, wound macrophages play a major role in wound healing by relasing multiple factors.[67] Angiogenesis factor (molecular weight 2000 – 20,000) is secreted by hypoxic macrophages[68,69] marginated at the wound edges, start neovascularization and granulation tissue formation. Deeper macrophages with adequate oxygenation, secrete growth factors which stimulate fibroblast proliferation, deposition of collagen, fibronectin and glycosaminoglycan.[70,71] Excessive bacterial prolifeation retards healing through release of excessive proteases. If the wound has not healed within 14 days, myofibroblast-sfibroblast bundles form and deposit excess collagen while mast cells release mucopolysaccarhides and histamine creating the basis for hypertrophic sear formation.

BROMELAIN

The pineapple plant, ananas comosus, has long been used for medicinal purposes. Native cultures used it as a digestive aid and as a remedy for skin disorders.[72] A compound called bromelain, which was found to be highly concentrated in mature pineapple stems[73] has since been linked to the medicinal properties. Research on bromelain has been conducted for decades in Europe and Asia, and in recent years it has been of interest in the United States. Although most of the available information comes from in vitro and animal studies or anecdotal evidence, rather than randomized, controlled clinical trials, bromelain has been shown to exhibit beneficial therapeutic effects while maintaining low toxicity and producing few harmful or undesired side effects. In particular, bromelain is reported to have anti-inflammatory, antiedematous, anticoagulant, and antimetastatic properties, and has also been shown to enhance antibiotic activity.[72,74,75]

WHAT IS BROMELAIN

Bromelain is crude, aqueous extract derived from pineapple stems and fruits. It is composed primarily of sulfhydrayl proteolytic exzymes that have protein –digesting and milk-clotting properties. Although there has been some controversy over the years as to whether the enzymes found in the stem and fruit are distinguishable as separate enzymes, current literature indicates that there are four distinct proteases in pineapples the two major enzymes are now described as stem bromelain and fruit bromelain. Several additional components have been found in bromelain, including peroxidase, acid phosphatase, several protease inhibitors, and organically bound calcium,[76] but their activities not well understood. Some studies indicate that proteolytic enzymes are not solely responsible for bromelain's pharmacological effects. Thus, further research is needed to determine whether the other components contribute to bromelain's medicinal properties.

COMMERCIAL AVAILABILITY AND RECOMMENDED USE

Bromelain is categorized as a food additive by the U.S.Food and Drug Administration and is on the list of substances generally recognized as safe.[77] The commercially available product is most often made from stem bromelain, whereby the extract is removed from cooled pineapple juice through centrifugation, ultrafiltration, and lyophilization and the remaining substance is made available to the public in the form of a powder, cream, tablet, or capsule. It is available in pure form or in multienzyme combinations (Debridase, Phlogenzym, and Traumanase).

Bromelain appears to be most effective when taken orally. In vitro studies have shown that low doses of bromelain are readily degraded by protease inhibitors in blood plasma and that oral administration may help bromelain retain its proteolytic activity.[78] Theoretically bromelain could also be degraded in the digestive tract; however the glycostylated nature of bromelain contributes to its functional stability[79] and may also prevent proteolytic degradation in the intestine. It has been suggested that bromelain be taken on an empty stomach, as it can interest with certain types of food.[80]

Recommended dosages of bromelain are available only through the scientific literature and vary depending on clinical indication. Most studies suggest 500 to 1500 mg/day taken in divided doses. Bromelain's activity is measured in gelatin digesting units, milk-clotting units. Federation Internationale Pharmaceutiques units. or Rorer units, and many manufacturers sell bromelain products that are standardized to 2000 gelatindigesting units in 500 mg tables.[80]

THERAPEUTIC BENEFITS AND MECHANISMS OF ACTION

Bromelain may be of particular interest in plastic surgery because of its apparent antiedematous, anti-inflammatory, and anticoagulation proprieties. Additional evidence suggets that bromelain may be beneficial in pain reduction, wound healing, burn debridement, and ischemia/reperfusion. It may even be an effective adjuvant to antibiotic therapy. The various mechanisms involved in these processes are just beginning to be understood.

Pain

Bromelain may be effective at reducing pain. In early studies, patients who were treated with bromelain experienced statistically significant decreases in pain associated with mediolaterial episiotomy[81] and in pain response to bradykinin that was topically applied to open blisters.[82] In a more recent study of mild acute knee pain in healthy adults, bromelain was shown to have a dose –dependent effect on the reduction of physical symptoms and improvement of general well being.[83] However bromelain was not effective and was no better than standard treatment. Placebo, or control in reducing pain associated with delayed onset muscle Soreness.[84]

Edems

Several studies have shown bromelain's antiedematous properties. In a double blind, place bo controlled study, bromelain was shown to reduce edema and ecchymoses in patients who experienced surgical (e.g. rhinoplasty) or nonsurigcal trauma to the face. The investigator suggested that the resolution of edema and ecchymoses in the bromelain treated subjects required one third to one half fewer days than would have been expected if these same patients has received placebo instead of active treatment.[85] In a study of ischemia/reperfusion injury in rabbits. Neumayer et al. observed reduced interstitial edema in rabbits treated with Phlogenzym (MUCOS Pharma GmbH & Co., Gerestried, Germany) a combination of bromelain. trypsin, and rutin, compared with rabbits that were not treated.[86]

Inflammation

There is evidence that bromelain acts as an anti-inflammatory agents. In a rat model of knee joint acute inflammation,inflammatory exudates from bromelain treated rats had reduced concentrations of the immung response.[87] Another mechanism of action relates to bromelain's ability to alter leukocyte expression of cell surface molecules. Hale et al. found that bromelain removes several types of cell surface molecules. thereby decresing leukocyte adhesion and activation ultimately resulting in decreased inflammation.[78]

Recent studies suggest that bromelain may be beneficial in treating inflammatory diseases. In a mouse model of inflammatory bowel disease, bromelain was found to decrease the clinical and histological severity of spontaneous colitis and colonic inflammation. While the proteolytic activity of bromelain seemed to be related to the reduction in inflammatory bowel disease symptoms., the exact mechanisms of action have yet to be determined.[88] Secor et al found that systemic bromelain treatment reduced the inflammatory process in a mouse and model of allergic airway disease.[89] In addition anecdotal evidence suggests that bromelain may be effective in treating mild ulcerative colitis. A 67 year – old woman and a 60 year old woman, both with ulcerative colitis, reported improved conditions after self-treatment with bromelain. In both cases, improvement of disease was confirmed by endoscopic examination.

Would Healing

Boirmelain may have beneficial in soft tissue wound healing. In a double blind, controlled clinical trial investigating the effects of bromelain on episiotomy wounds. Howat and lewis reported faster rates of reduction of edema and bruising in subjects who received bromelain treatment compared with subjects who received placebo.[90] The authors noted, however, that none of the results reached statistical significance. In a study of wound healing in healthy adults, an oral nutritional supplement containing bromelain, other protease the soft tissue would healing time when administered during the early phase of wound healing. However, it is important to note that the efficacy of each component of the supplements was not determined, and it is unknown if the components had an additive or synergistic effect.

Platelet Aggregation

Several earlier studies have suggested that bromelain may be an effective anticoagulant. In vitro and in vivo studies[93,94] have shown bromelain to reduce adenosine 5 diphosphate induced platelet aggregation by degrading fibrinogen. Bromelain was more effective at degrading purified fibrinogen rather than fibrinogen in plasma,[94] possibly because of the action of protease inhibitors in the plasma. In a more recent study, Glaser and Hiberg report that bromelain decreased adenosine 5 diphosphate – and thrombin receptor – activated peptide – 6 induced platelet aggregatioin, most likely by altering fibrinogen receptors and blocking the formation of frbrin.

Antibiotics

Bromelain has been shown to enhance the action of antibiotics. In an early study, bromelain was found to increase tissue permeability to antibiotics, although the results were not statistically significant. Tinozzi and Venegoni found a statistically significant[95] increase in serum and tissue levels of amoxicillin in subjects treated with the antibiotic and bromelain.[96] More recently, bromelain was shown to be effective at enhancing the activity of antibiotics inchildren with sepsis. Shahid et al., observed a statistically significant decrease in the number of days it took for fever to subside and for the withdrawal of hemodynamic support in the children who received phlogenzym with antibiotics compared with the children who received antibiotic therapy alone.[97]

Burn Debridement

Bromelain may be an effective alternative to surgical escharotomy in patients with deep burns. Results of in vitro and in vivo studies show that bromelain preparations can effectively debride full-thickness burns in pig skin in less than 24 hours.. The preparations affected only burned skin and resulted to minimal blood loss.[98] Debridase (Biotechnology General L.TD. Kiryat, Malchi, Israel). a bromelain-derived preaparation, has also been shown to be an effective burn-debriding agent. In a preliminary study of 130 patients with deep second and third degree burns, Rosenberg et al. found that, in most cases treatment with Debridase resulted in complete debridement of eschar after only one or two brief applications; however, the investigation note that data were incomplete for a large number of patients, and the non comparative nature of the study did not allow for a comparison between debridase and standard of care.[99] In a procine model of burn induced compartment syndrome, circumferential limb burns treated with Debridase exhibited a statistically significant reduction in intra compartmental pressures compared with untreated burns and enzymatic digestion of burn eschar effectively cleaned the wound area without damaging viable tissue.[100]

Ischemia/Reperfusion

Neumayer et al., found that Phlogenzym had a protective effect on skeletal muscle during ischemia/reperfusion studies in rabbits. The authors suggest that bromelain's ability to reduce platelet and leukocyte aggregation may have reduced clotting in the microvessels, thereby preventing the no-reflow phenomenon.[86]

Toxicity and Side Effects

Bromelain has been shown to have low toxicity. In several animal studies, the median lethal dose during oral administration was greater than 10g/kg. When bromelain was administered intravenously and intraperitioneally, the median lethal dose ranged from 20 to 35 mg /kg and from 36 to 85.2 mg/kg respectively.

Most studies of bromelain report a low incidence of adverse effects. In a review of clinical studies that investigated bromelain’s effect on osteoarthritis, no serious adverse events were reported however, there were some cases of gastro intestinal problems, headache, tiredness, dry mouth, skin rash, and unspecified allergic reactions. In these studies, bromelain was administered at dosages ranging from 540 to 1890 mg/day. Higher dosages of bromelain tended to have higher incidences of adverse drug reactions compared with standard treatment.[101]

Other investigators have reported isolated cases of allergic reaction and exacerbation of asthma symptoms as a result of occupational exposure to bromelain. In most cases, adverse reactions occurred after inhalation of bromelain: however some patients experienced gastrointestinal discomfort after per oral challenge with pineapple. The respiratory and gastrointestinal symptoms in these cases were found to be the result of immunoglobulin-E mediated reactions to bromelain.

Some authors have suggested that bromelain’s anticogulant properties could increase bleeding when it is taken in combination with other medications, such as aspirin and warifarin.[105]

Despite the few reports adverse events, bromelain is generally considered to be safe, but it is important to note that most of human studies involving bromelain included adult subjects. Thus there is little information on the safety of bromelain for children younger than 18 years of age. In addition, little information is available on the safety of bromelain when it is administered at higher doses, when it is taken in combination with other medications, or when it is taken long term.

FUTURE RESEARCH

Although bromelain has been studied for decades in Europe and Asia and more recently in the United States, most of the available literature desecribes results of in vitro or animal studies. Very few randomized, controlled clinical trials have been conduced. In order for bromelain to be widely accepted as a therapeutic agent more trials are needed to establish the efficacy and optimall dosage for each clinical indication. In addition, since many bromelain preparations contain other enzymes and substances, more research is need to identify bromelain's contribution to the therapeutic effects of these products.

CONCLUSIONS

The pineapple compound bromelain has long been used for its medicinal properties.,Although the mechanisms of action are just beginning to be understood, many studies have suggested that the proteolytic component of bromelain is primarily responsible for the pharmacological effects. Bromelain may be of interest to plastic surgeons because of its apprarent ability to reduce pain, edema, inflammation and platelet aggregation, as well as its ability to potentiate antibiotics, which may be beneficial in postoperative healing. Bromelain's reported efficacy in burn debridement and ischemia/reperfusion may also have positive applications in plastic surgery. Although bromelain is widely used and generally considered to be a safe substance, more randomized, controlled clinical trials are necessary to further elucidate its clinical potential.

Surgical Management of Burn Wounds

The Procedures used in the surgical management of burns can be classified as follows.

1. Management of partial thickness burns:

    a) Tangential Excision and Split Skin Grafting

    b) Tangential Excision and biological Dressing

    c) Superficial Escharectomy.

2. Management of full thickness burns

    (a) Escharotomy

    (b) Radical Excision

    (c) Sequential Excision

MATERIALS AND METHODS

PLACE OF STUDY

This is a prospective, non-comparative study involving 25 patients both mate and female admitted to the burn unit, Department of Burns, Plastic and Reconstructive Surgery, Kilpauk Medical College,Chennai.

PERIOD OF STUDY

Jun 2005 – December 2006

STUDY GROUP

25 patients both male and female of age group 15 years to 50years with burns of total body surface area of 10-40% were included in the study. These patients had burns ranging firm second degree deep to fall thickness burns.

CRITERIA

1. Patients were excluded in the presence of severe smoke inhalation, a recent history of myocardial infarction, concurrent acute injury (or) disease that might compromise the patient's life or welfare. Significant hematological, cardiovascular, hepatic (or) neoplastic disease or other immediate life threatening conditions.

2. Patients were also excluded if they had poorly controlled diabates mellitus, a history of allergy atopic disease or known sensitivity to pineapple (or) were pregnant (or) nursing.

3. Pre and post application investigations like TC, Hemoglobin, liver function tests like SGOT, SGPT, SAP, Serum creatinin were done to rule out any adverse effect.

ENZYMATIC AEBRIDEMENT WITH ESCHYLASE

Each burn wound was cleaned with saline soaked gauze and covered with Heparin soaked gauze for 24hrs. After removing the dressing reassessment of the burn's depth was made. Deep dermal (or) full thickness wounds were covered with a mixture of eschalyse gel prepared at a concentration of 2g enzyme in 20-40 g hydrating carrier gel for every 10 m x 10c m of eschar.

The burn wound were then covered with an occlusive dressing for a period of up to 24 hrs. During this period the patient was closely observed for the presence of pain or itching and pain relieving medication, were given as necessary.

Twenty four hours after 1st application occlusive dressing were removed aseptically and the entire area wiped clean using a wooden tongue depressor, dry gauze and later with saline soaked gauze until the appearance of a clean, bleeding surface (or) until no further eschar be removed.

After wiping the treated area the wounds were reassessed and covered with a saline soaked absorbent dressing for another 24 hours. It at this point debridement was deemed still unsatisfactory, Eschalyse gel was reapplied.

Treatment following Eschalyse application

Following debridement full thickness burns were graffed with split thickness graft.

Deep burns with dermal remnants or superficial burns were treated conservatively with topical antimicrobial agents (or) Heparin saline dressing and sometimes with thin split skin graft.

Outcomes:

The primary and points of this study

1. Extent of achieved debridement
2. Number of Eschalyse applications required
3. Presence of adverse events such as pain, itching, fever, local and systemic infection.

a. Visuall assessment of debridement efficacy was assessed by our Head of the Department and by another chief and estimating the amount of original eschar that was removed according to the following classification.

Excellent	-	85 – 100%
Good	-	70 – 85%
Fair	-	60 – 69%
Poor	-	50 – 59%
Failure	-	< 49%

In case where additional eschalyse was applied the debridement efficacy was assessed using the amount of eschar that was removed in each application.

All the adverse events were recorded during hospitalisation and based on past experience with other enzymatic debriding agents fever and pain was especially important.

RESULTS

INCIDENCE OF AGE AND SEX IN THE BURNS CASES

Age group in year	Male	Female	Total
10 – 20	3	-	3
21 – 30	4	7	11
31 – 40	6	2	8
41 – 50	2	1	3
Total	**15**	**10**	**25**

Our study include mainly patients between ages 20 – 40 years. Children were excluded because of ethical reasons. There was a slight preponderance towards male 15 Males(60%) , 10 Females(40%).

CAUSES OF BURNS

	Male	Female	Total
Flame	9	7	16
Scolds	4	3	7
Contact	1	1	2
Total	14	11	25

Most of our enzyme application was done in flame burns 16cases(64%) and Scalds 7cases (28%) and we have not applied in electrical burn involving the limbs and the flash burns was included in flame burns itself and here the contact burns 2 cases (8%) implies to acid burns.

TOTAL BURN SURFACE AREA

Most burns 64% covered < 10% of total burn surface area. Burns covering 10 – 40% total burn surface area accounted for 32% of cases and burns covering >40% total burn surface area accounted for 4% of cases.

% of Surface	Male	Female	Total
< 10%	9	7	16
11 – 40%	4	4	8
> 40%	1	0	1
Total	14	11	25

TYPE OF BURNS

15 cases (60%) of them were of mixed type

8 cases (32%) of them were Deep

2 cases (8%) of them were full thickness

	Mixed	2' Deep	Full thick
Male	10	3	2
Female	5	5	
	15	8	2

DISTRIBUTION OF BURNS

Upper limb - 14 case (56%)

Trunk - 5 case (20%)

Lower limb - 4 case (16%)

Neck - 2 case (8%)

	Upperlimb	**Trunk**	**Lower limb**	**Neck**
Male	7	4	2	-
Female	7	2	2	2
Total	14	6	4	2

NO. OF APPLICATIONS

Single Application - 7 cases 28%

Two Applications - 12 cases 48%

Three Applications - 6 cases 24%

	Single application	**Double application**	**Three application**
No. of cases	7 cases 28%	12 cases 48%	6 cases 24%

PERCENTAGE OF DEBRIDEMENT

After 1st Application - 70 - 80%

After 2nd Applications - 80 - 90%

Type of burns	**1st application**	**2nd application**	**3rd application**
Mixed	70 – 80%	80 – 90%	-
2nd Degree deep	70 – 80%	80 – 90%	95%
Full thickness	70 – 80%	80 – 90%	95%

MOST COMMON ADVERSE EVENTS

That were noted are

Fever

Localised Pain

Burning sensation

DISCUSSION

The concept of debridement is as old as medicine itself. The first reference to debridement appears in the old testatment. The prophet Isaiah is quoted. Take a bunch of figs they took and placed the figs on the ulcer the Hezekiah recovered. Christopher Columbus also described the use of pineapple juice to promote healing.[106] Although the importance of the debridement is well-accepted, there is no clear definition of how much healthy tissue should be sacrificed in order to achieve adequate debridement. The ideal debridement method should consists of topical agent that results in rapid, safe, and effective debridement of necrotic tissue with little or any damage to healthy tissue.

Chemicals with debriding activity such as acetic acid[107] pyruvic acid[108,109] phosphoric acid[110,111] salicylic acid, benzoic acid, mallic acid (ascerbine)[112,113] trypsin[114,116] and fibrinolysin – desoxyribonuclease[117,118] were or still are in current clinical use ever since the second world war. These compounds require one or two daily dressing changes for 5-10 days. As a result, by the time the eschar is removed, a rich granulation tissue has already developed with the prospect of future scarring and contracture formation.

Several enzymes of microbial, vegetable or even animal origin have been tested with some even reaching the market. These enzymes include those derived from microorganisms such as Clostridium histoliticum H-4: Collagenase (Santyl C)[120,124], Bascillus subtilis : (Subtilains (C), Streptococci: Streptokinase – streptodornase (Varidase)[125,126] as well as from plants such as the papaya (papain – urea: Accuzyme C)[127] or bromelain from the pineapple.[14,16,18,127,135] Even enzymes made from krill, blowfly larvae extracts, or pancreatic extracts have been tried [106,136].

Presently only the collagenase: Varidase – Santyl (C), Papain urea: Accuzyme and Salicyclic acid are used, relatively rarely in the field of burns and acute trauma. Unfortunately these slow acting agents require several daily dressing changes for no less than five and sometimes more than 12 days to be effective or end up with an incomplete debridement that necessitates additional surgical debridement. The repeated application of these agents has been followed in several cases by local and systemic infection due to bacteremia secondary to the repeated handling and occlusion of the contaminated eschar with exposure of the surrounding raw tissues to the contaminated the partially dissolved eschar.[10,13]

The aim of the current study was to evaluate the efficacy of burn wound debridement using the Eschalyse enzymatic preparation as well as to evaluate the presence of any possibly related adverse events. Direct assessment of the debridement thoroughness (efficacy) and its safety was sought as the primary end points and not graft take as many factors besides the host bed influence the graft take as many factors besides the host bed influence the graft take. Another reason for not including graft take as a measure of debridement efficacy is the fact that selective enzymatic debridement removes only the necrotitc tissues and deep but not full thickness burns may still heal by epithelialization of the surving dermal bed from the epidermal adnexae without the need for transplantation surgery. This differs significantly from surgical debridement where in most cases the full thickness of the skin in sacrificed for the sake of debridement and transplanation of a skin graft is mandatory.

The study population is fairly typical of our burn unit population, though slightly more representative of adults since judicious inclusion of children in the study was encouraged. The mean BSA of the deep burns that required debridement and were treated by was less than 7% of BSA. The majority of burns were debrided using two applications of Eschalyse. In the remainder of cases for which two or more applications were required the wound was usually old, dry or saturated with silver sulfadiazine (SSD). A pressure contact burn of the fingers and the other of Old SSD treated scaled burn of the arm, three consecutive applications were required.

In most cases the debridement was completed in less than 5 day. In average, the first application removed nearly 75% of the entire eschar, the second (if needed) 85% and in the few cases in which a third application was used, 95 of the eschar was eventually removed. In nearly three quarters of the cases two 24 hour application was sufficient with a gap of 24 –36 hours to complete this phase of the treatment.

In one case of Acid burns involving thigh grading to full thickness even after three applications we were not able to remove the eschar except it become loose and we dont get a good result or failure in acid burns and the exact reason not known.

During the early phases of the study many of the debrided wound beds that had typical features of a non surgical debrided bed were misinterpreted only as partially debrided, skewing the results against. Once it became clear that this bed was clean and could heal spontaneously or support a skin graft the results “improved”. In some difficult areas (such as on the trunk or on convex areas where the debriding gel could not be in close and continuous contact with the eschar) additional applications were required to complete the debridement of the entire intended area. Early on the importance of removing all the superficial burned keratin layer (blisters) that hinder the action of the enzymes was realized. Dry and old eschars, especially if treated with SSD for several days, did not dissolved as well as fresh most ones. Thus the efficacy of Debridase decreased after the 6^{th} post burn day possibly due to the changing nature of the eschar following treatments such as SSD.

Five patients with extensive buras were treated several times, each time for no more than 15% TBSA of the burned area. In such cases eschalyse dressings were applied sequentially after removing the previously debrided dressings.

Approximately one quarter of the burns were completely debrided within the first day with another half undergoing complete debridement within 2-3 days of injury. These results are even more impressive when one considers that in some cases (e.g., more extensive burns) sequential applications of eschalyse were used. In all cases where enzymatic debridement with eschalyse was complete, skin allografting, for biological, epithelialization enhancing cover or other topical covers were performed at the bedside with minimal pain and no bleeding. In cases of full thickness burns and an excellent eschalyse debridement the grafting of an autograft could be done without a farther surgical debridement and without the repeated blood transfusions in case of repeated surgery and furthur grafting the recipient bed prepared by scraping or dermabrasion.Total duration of stay in the hospital were drastically reducedand seems to be very cost effective. Psychological and rehabilitative part on behalf of the patient remains easy. This is obviously of great benefit when compared to the pain and bleeding associated with surgical debridement, The selectivity of the preparation was demonstrated by the lack of effect on the healthy surrounding tissue and the preservation of remnants of unburned tissues (dermis and epidermis).

Finally, unlike other non surgical debridement techniques, eschalyse achieved a thorough debridement in a single or double application offering a rapid and selective burn wound debridement method.

Many patients complained of various sensations at the treated area immediately on application on eschalyse. These sensations ranged from a vary mild tingling or discomfort to a mild burning or itching with some requiring oral analgesia mainly with the more superficial burns. Most of these complaints subsided within 30 min of eschalyse application, but some patients complained of an itching or burning sensation for the entire duration of the treatment. Typically, the more intense sensations started to decrease after 15-20 min of treatment reaching a level very mild irritation within 30 min of application. Prophylactic pre-treatment of patients with analgesic agents (as is customary before dressing changes) usually was sufficient to keep the patients comfortable. None of our patients required sedation or general anesthesia for the treatment.

Fever was noted in nearly 80% of our patients, however fever is common after thermal injuries and the exact relationship to the debridement is unclear. It should be noted that 2/3 of our patient population did not develop any fever beyond the first 2 days after injury when in general fever becomes more common. Also we did not find that the temperatures of patients treated with eschalyse were any higher than in our other burn patients during the study period that were not treated with enzymatic debridement. Only a Large prospective randomized comparative trial will help determine the relationship between enzymatic debridement with and the occurrence of fever.

Our study has several limitations that merit further discussion. Further –more this was not a comparative trial, thus it is unclear what the exact impact of enzymatic debridement compared to the standard of care tangential excision was.

SUMMARY AND CONCLUSIONS

- 25 patients with 10-40% burn admitted to tertiary care center were included in the study.

- Majority of the patients affected were 20-30 years of age.

- Children were not included in the study.

- There was a slight male preponderance 15 cases(60%) in our study population.

- Commonest cause of burns included in the study were flame burns16cases(64%), followed by scalds(28%), and contact burns 2cases(8%).

- Patients with <10% of total burn surface area formed 64% of the study population.

- Patients with myocardial infarction, concurrent acute injury, significant haematological, cardiovascular, hepatic diseases were excluded.

- Bromelain is a well known group of enzymes extracted from pineapple fruits (or) stems. It contains more than 50 different components and is widely used as an over the counter food additive and is also used in the cosmetic industry.

- Most burns included in our study were of mixed burns 15 cases (60%), while 8 cases(32%) were of deep dermal burns.

- Majority of the burns included in our study involves upper limb 14cases (56%), Trunk 5 cases (20%), Lowerlimb 4 cases (16%) & Neck 2cases(8%).

CONCLUSIONS

- On analyzing our result we achieved debridement with single application in 28% of cases, and two applications in 48% of cases and three applications in 24% of cases.

- Percentage of debridement achieved with single application is 70± 10% and after two applications we achieved 80±10% and after 3 applications we achieved around 95 ± 5%.

- Approximately one quarter of the burns were completely debrided with in one or two days and another half complete debridement with in 3-4 days and in some extensive burns sequential application helps us to achieve the debridemnet in a short time.

- . In all cases where enzymatic debridement with eschalyse was complete, skin allografting, for biological, epithelialization enhancing cover or other topical covers were performed at the bedside with minimal pain and no bleeding.

- . In cases of full thickness burns and an excellent eschalyse debridement the grafting of an autograft could be done without further surgical debridement and without the repeated blood transfusions in case of repeated surgery and furthur grafting the recipient bed prepared by scraping or dermabrasion. This is obviously of great benefit when compared to the pain and bleeding associated with surgical debridement and the patient not subjected to repeated anesthesia.

- The selectivity of the preparation was demonstrated by the lack of effect on the healthy surrounding tissue and the preservation of remnants of unburned tissues (dermis and epidermis). Finally, unlike other non surgical debridement techniques, eschalyse achieved a thorough debridement in a single or double application offering a rapid and selective burn wound debridement method

- Total duration of stay in the hospital were drastically reduced and seems to be very cost effective.

- Psychological and rehabilitative part on behalf of the patient remains easy.

- Fever, localized pain and burning sensation were observed as side effects and managed appropriately. No specific morbidity and mortality noted.

- To conclude the availability of a rapid, reliable and complication free enzymatic debriding agent may open new horizons and provide a new treatment modality for burn wound management.

APPENDIX

Eschalyse gel

Mixture of eschalyse powder and carrier hydrating gel at a Concentration of 2g enzyme in 20-40g hydrating carrier gel for every 10cm x 10 cm of eschar.

Heparin Saline Dressing

1 vail containing 5000 IU in 100ml of normal Saline used for covering 10% of burn surface area.

PROFORMA

Name : D.O.A :

Age : D.O.D:

Sex : IP NO :

Occupation : Ward No:

Education : Weight :

Marital Status: Time of incident :

Time of Starting treatment:

Type of Burns: ☐ Flame ☐ Scalds ☐ Contact

Degree of the burns:

Depth of the burns : Superficial ☐ Deep ☐

Sites Affected :

Sites Affected

Head

Neck

Anterior trunk

Posterior trunk

Right buttock

Left buttock

Genitalia

Right upper arm

Left upper arm

Right hand

Left hand

Left thigh

Right leg

Left leg

Right foot

Left foot

No. of Applications

% of debridement achieve after

Ist Application

2nd Application

3rd Application

ABBREVIATION

SGOT	:	SERUM ALANINE AMINOTRANSFERASE
SGPT	:	SERUMASPARTATEAMINO TRANSFERASE
SAP	:	SERUM ALKALINE PHOSPHATASE
TC	:	TOTAL COUNT
TBSA	:	TOTAL BURN SURFACE AREA
SSD	:	SILVER SULFADIAZINE
PMN	:	POLYMORPHONUCLEAR LEUCOCYTES
PG	:	PROSTAGLANDINS
SOD	:	SUPEROXIDE DISMUTASE
NK	:	NATURAL KILLER CELLS
NADPH	:	NICOTINAMIDE ADENINE DINUCEOTIDE PHOSPHATE DEHYDOGENASE

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